Green Extraction and Liposomal Encapsulation of Grape Pomace Polyphenols for Nutraceutical Use

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ABSTRACT:

Grape pomace (GP), a by-product of grape processing, is rich in polyphenolic compounds with significant antioxidant, anti-inflammatory, and antimicrobial properties. This study aimed to enhance the extraction and encapsulation of GP polyphenols to improve their stability and bioavailability. Polyphenols were extracted using a synergistic combination of soaking and ultrasound-assisted extraction (UAE), achieving the highest antioxidant activity (250.76mg TE/g DM) and total phenolic content (101.10 mg GAE/g DM) at a 10% GP concentration. Liposomes encapsulating these extracts were prepared and characterized for their physicochemical properties. The liposome formulations exhibited a particle size range of 83.6–111.1 nm, zeta potential values around -23 mV, and encapsulation efficiencies up to 69.73%. The results demonstrate that combining soaking and UAE maximizes polyphenol recovery while encapsulation in lecithin-based liposomes provides a stable delivery system for these bioactive compounds. This approach underscores the potential of grape pomace valorization for developing functional foods and nutraceuticals.

Keywords: Grape pomace; Antioxidant activity; Phenolic compounds; Encapsulation efficiency.

INTRODUCTION

Grape pomace (GP), a by-product of grape processing, is a rich source of polyphenolic compounds, including flavonoids, phenolic acids, anthocyanins, and tannins. These bioactive compounds are widely recognized for their potent antioxidant, anti-inflammatory, and antimicrobial properties, making GP an attractive candidate for the development of functional foods, nutraceuticals, and pharmaceuticals (Vašeková et al., 2020; Karastergiou et al., 2024). However, the practical application of these compounds is often hindered by their low stability, susceptibility to environmental degradation, and limited bioavailability under physiological conditions (Gharby et al., 2022).

Efficient extraction techniques play a crucial role in maximizing the recovery of polyphenols from GP. Among these, ultrasonication has gained prominence as a green, cost-effective, and scalable method. Ultrasonication enhances extraction efficiency by generating acoustic cavitation, which disrupts cell walls, facilitates solvent penetration, and releases intracellular bioactive compounds. This technique operates under mild conditions, reducing the risk of thermal degradation of heat-sensitive compounds, and is particularly effective when combined with soaking to optimize yield and preserve compound integrity **(Chemat et al., 2020)**. By improving extraction efficiency and selectivity, ultrasonication contributes significantly to the sustainable utilization of GP for high-value applications.

To further address the challenges of stability and bioavailability, encapsulation technologies have been widely applied to protect and deliver bioactive compounds. Liposomes, nanoscale vesicles composed of lipid bilayers, are particularly effective for encapsulating hydrophilic and hydrophobic compounds. These systems enhance the stability of sensitive bioactives, improve controlled release, and increase bioaccessibility (Aanniz et al., 2024; Jalali-Jivan et al., 2022). This study aims to develop nano- secondary liposomes encapsulating polyphenolic extracts from GP using chitosan and maltodextrin as secondary-layer materials. Polyphenols were extracted using a combination of ultrasound-assisted extraction and soaking, leveraging the synergistic effects of these methods to maximize yield and preserve antioxidant properties. The prepared nano-secondary liposomes were characterized for their particle size, zeta potential and encapsulation efficiency

MATERIALS AND METHODS

Plant material and reagents

Fresh grape pomace was generated by juice manufacturing and consisted of skins, seeds and rest of pulp. It was kindly provided by Egyptian juice shops in Cairo, Egypt, where

grapes were cultivated and processed. The material was dried in a forced air dryer under 50 °C up to constant weight, then it was milled in a knife miller to get a powder. Soy lecithin granules were obtained from Solgar, Inc. (Leonia, NJ 07605, USA). Methanol (≥99.9%), ethanol (≥99.8%), sodium hydroxide (NaOH, ≥97.0%, pellets), Gallic acid (≥98.0%), Folin & Ciocalteu's phenol reagent, sodium carbonate ($Na_2CO_3 \cdot H_2O$, ≥99.5%), hydrochloric acid (HCl, 37%), and 2,2-diphenyl-1-picrylhydrazyl (DPPH), were supplied by El-Gamhouria Trading Chemicals and Drugs Company, Egypt.

Methods

Extraction process

Phenolic compounds were extracted from GP powder using the soaking (S) method under varying GP powder -to-solvent ratios. GP powder was weighed and mixed with the solvent (70% ethanol) at ratios of 2%, 4%, 6%, 8%, and 10% (w/v). The prepared mixtures were placed in sterile glass containers and stirred gently to ensure complete contact between GP powder and the solvent. The containers were covered to prevent solvent evaporation and left to soak at room temperature (approximately 22–25°C) for 24 hours. After the soaking period, the mixtures were centrifugation at 8,000 x g for 10 minutes to separate the liquid extract from the plant residue. The filtrates were collected in amber bottles to minimize light exposure and stored at 4°C for further analysis.

A laboratory-scale ultrasonic probe sonicator (UPS, Sonics, Vibra Cell) operating at a frequency of 20 KHz, power of 200 W, and amplitude of 70% was employed for the extraction of phenolics by ultrasound-assisted extraction (UAE), following the method outlined by Tabaraki et al., (2012). Different ratios of GP powder (2,4,6,8 and 10% w/v) were suspended in an extraction solvent (70% ethanol) and stirred thoroughly to ensure uniform penetration. The extraction mixture was placed in glass beakers and maintained in an ice bath. The probe tip was inserted 2 cm into the sample solution and subjected to sonication for durations of 10 minutes. To mitigate the heat generated during sonication, an ice bath was utilized to maintain the extraction mixture temperature at 40°C. The resulting extract underwent centrifugation at 8,000 revolutions for 10 minutes. The filtrates were collected in amber bottles to minimize light exposure and stored at 4°C for further analysis.

S samples which combined both UAE was produced from GP. For this, firstly S was applied at the same conditions and followed by the conditions of UAE.

Determination of Antioxidant Activity (AA) and total phenolic content (TPC)

TPC and AA were measured in GP extract (GPex). AA was assessed using the DPPH method based on the methodology described by Chen et al., (2013) with modifications. The activity of extract to scavenging the radical DPPH was expressed based on the Trolox calibration curve ($R^2 = 0.9986$), as mg of Trolox equivalent (TE) per g of flour of peel or seed. A 0.079 M DPPH solution in methanol was prepared with an absorbance between 0.700 and 0.900 ($\lambda = 517$ nm). The sample extract was mixed in relation (1:29 µL) with DPPH solution, and then stored in darkness for 30 min at 24 °C. Subsequently, the absorbance was measured using a UV/vis Scanning Spectrophotometer at 515 nm. The cero standard was set with methanol, and results were expressed as mg trolox (TE)/g DM. The DPPH radical scavenging was performed in triplicate.

The Folin-Ciocalteu method (Kim et al., 2003) was used for TPC determination. GPPex was mixed with 0.8 mL of 0.2 N Folin-Ciocalteu reagent and 0.8 mL of a 20% Na_2CO_3 solution. After 2-hour incubation at 25°C, the absorbance was measured at 765 nm using a UV-Vis spectrophotometer (Shimadzu, Japan). TPC was calculated using a standard curve prepared with Gallic acid standards ranging from 0 to 100 mg/L. Samples were analyzed in triplicate, and the results were expressed as milligrams of Gallic acid equivalent (GAE) per grams of sample.

Preparation of liposome

A lecithin dispersion (2%, w/w) in acetate buffer (pH 3.5 ± 0.1; 0.1 M) was prepared. Then, freeze dried GPex (0.2, 0.4, 0.6, 0.8 and 1 w/v) was dissolved in lecithin solution. For homogenization of the lecithin dispersion, ultrasonication probe (frequency of 20 KHz, power of 200 W, and amplitude of 70%) was used for 5 min to obtain liposomes.

Characterization of GPex loaded liposome

The surface charge (ζ-potential), particle diameter (Z-average) and polydispersity index (PDI) of GPex loaded liposome were evaluated using the dynamic light scattering (DLS) method (Zetasizer Nano ZS, Malvern Panalytical Ltd, Malvern WR14, UK). Samples were prepared via dilution of an adequate

volume of approximately 0.1 mL, which was added to approximately 3.5 mL of distilled water. Then, the mixture was subjected to gentle sonication. The diluted sample was transferred to a 3 mL disposable PVC transparent cuvette and a 1 mL two electrode PVC disposal cell for zeta potential measurements. For each sample, a detection angle of 173° was chosen for the size measurement unless stated otherwise. The refractive index of the sample was approximately 1.47, and the optical absorption was adjusted to an absorbance of 0.1.

Encapsulation efficiency (EE)

According to Montoro-Alonso et al., (2024), the liposomes were centrifuged (12,000×*g*, 180 min, 20 °C), the supernatant was separated and analyzed, and the pelleted liposomes were resuspended in fresh distilled water. These resuspended liposomes were disrupted by adding 1 mL methanol and 1 mL chloroform to determine the amounts of encapsulated compounds. The mixture was vortexed thoroughly and left to allow phase separation. The concentrations of phenolics in the upper water–methanol phase and in the supernatant were determined and considered as the encapsulated and nonencapsulated fractions, respectively. The encapsulation efficiency was calculated using Eq. (1):

$$\mathbf{EE}\ (\%) = \frac{Mass\ of\ encapsulated\ phenolics}{\text{Total mass of phenolics(encapsulated + nonencapsulated)}} \times \mathbf{100}$$

RESULTS AND DISCUSSION

Antioxidant Activity and Total Phenolic Content of GPPex

The choice of extraction method significantly impacted both the antioxidant activity and total phenolic content of grape pomace extracts. Among the assessed methods, traditional soaking exhibited the lowest efficiency (Siah et al., 2014). This may be attributed to its inability to effectively disrupt plant cell walls, limiting the release of bound phenolic compounds (Rousseau et al., 2020). In contrast, ultrasonication significantly enhanced the extraction efficiency. The ultrasonic process generates cavitation bubbles, which collapse and create localized high pressure and temperature, facilitating the breakdown of cell structures and the release of intracellular components (Fu et al., 2021). These findings align with previous reports that highlight ultrasonication as a superior technique for enhancing phenolic recovery and antioxidant activity from plant matrices (Rao et al., 2021; Guo et al., 2024; Liao et al., 2015; Huang et al., 2022).

The combined method, which integrates soaking as a pre-treatment with subsequent ultrasonication, yielded the highest antioxidant activity and total phenolic content across all concentrations. At 10% GPPex, this method achieved 250.76 mg TE/g DM and 101.10mg GAE/g DM for antioxidant activity and total phenolic content, respectively. This result underscores the synergistic effect of combining soaking, which preconditions the plant matrix, with ultrasonication, which maximizes cell disruption and phenolic release (Zheng et al., 2024; Fu et al., 2021). This two-step approach has been validated in previous studies as an effective strategy for enhancing the extraction of bioactive compounds.

A concentration-dependent increase in both antioxidant activity and total phenolic content was observed across all extraction methods. Higher concentrations of grape pomace provided a greater reservoir of bioactive compounds, leading to improved radical scavenging capacity. The combined extraction method consistently outperformed the individual methods, demonstrating superior efficiency in extracting phenolics at all concentrations. At 2% concentration, the total phenolic content achieved using the combined method was 73.61 mg GAE/g DM, significantly higher than that obtained with soaking (34.52 mg GAE/g DM) or ultrasonication alone (54.37 mg GAE/g DM). The enhanced performance of ultrasonication and the combined method can be attributed to their ability to overcome physical barriers within the plant matrix. Ultrasonication disrupts cell walls through mechanical and acoustic forces, while the soaking pre-treatment softens the plant material, facilitating the release of bound phenolics. The findings emphasize the importance of adopting advanced extraction techniques that integrate pre-treatment steps to maximize bioactive compound recovery (Khadhraoui et al., 2021).

Characterization of GPex loaded liposome

Particle size, PDI & zeta potential

The incorporation of grape pomace phenolic extract into lecithin-based liposomes significantly influences the physicochemical properties of the resulting nanoparticles, as observed in terms of particle size, polydispersity index (PDI), and zeta potential. These findings align with existing literature on the interaction of phenolic compounds with

lipid bilayers and their encapsulation behavior.

The control liposome exhibited a particle size of 105.8 nm, which increased slightly to 111.1 nm with the addition of 0.2% phenolic extract (L1). This initial increase can be attributed to the integration of phenolic molecules into the lipid bilayer, causing structural expansion, as previously reported in studies on phenolic-lipid interactions (Saroglu et al., 2024). However, as the concentration of the phenolic extract increased (L2 to L5), the particle size decreased nonlinearly, reaching a minimum of 83.6 nm at 1% phenolic concentration (L5). This trend suggests that higher phenolic concentrations enhance lipid bilayer packing efficiency, reducing particle size due to improved encapsulation and stabilization, consistent with findings from nanoencapsulation of phenolic compounds (Yanagihara et al., 2023). The observed nonlinear behavior highlights the dual role of phenolic compounds as both stabilizers and potential disruptors at intermediate concentrations (e.g., L3). At 0.6%, partial aggregation may occur, leading to a transient increase in particle size, which stabilizes at higher phenolic concentrations. This phenomenon has been noted in studies where phenolics exhibit concentration-dependent effects on lipid membrane organization (Frøkjaer et al., 2019).

The PDI values for all formulations remained within the acceptable range (<0.3), indicating that the liposome populations were monodisperse and uniform. These results suggest that phenolic extracts do not significantly compromise the size distribution of liposomes, which is critical for ensuring consistent bioactivity and delivery properties. The slight variations in PDI between formulations (e.g., L2 at 0.276 versus L3 at 0.253) may reflect minor differences in phenolic distribution within the lipid bilayer (Fan et al., 2021).

The zeta potential of the control liposome was -23.3 mV, which decreased slightly with the incorporation of phenolic extracts. The least negative value (-19.7 mV) was observed at the lowest phenolic concentration (L1), potentially due to partial neutralization of surface charges by phenolic molecules. However, as the phenolic concentration increased (L2 to L5), the zeta potential stabilized around -22 to -23.5 mV, indicating that higher concentrations of phenolics do not significantly alter surface charge. These results are consistent with the literature, which suggests that phenolic compounds can either mask or enhance surface charge depending on their concentration and interaction with the lipid bilayer (Giordani et al., 2023).

The results demonstrate that grape pomace phenolic extracts can be effectively encapsulated in lecithin-based liposomes without compromising stability or uniformity. The reduction in particle size at higher phenolic concentrations (L4 and L5) is particularly advantageous for enhancing bioavailability and cellular uptake, as smaller liposomes are more readily absorbed and penetrate biological barriers. Moreover, the stable zeta potential values suggest that these formulations are electrostatically stable, reducing the likelihood of aggregation during storage and application.

***Encapsulation* efficiency**

The encapsulation efficiency (EE) results for the liposomal formulations loaded with grape pomace phenolic extract (L1 to L5) indicate that the phenolic extract concentration plays a crucial role in determining the effectiveness of encapsulation. As the concentration of the phenolic extract increases from 0.2% (L1) to 0.6% (L3), EE steadily increases, reaching a maximum of 69.04% at L3, with a slight plateau at 69.73% for L4 (0.8%). However, at the highest concentration (L5, 1%), EE decreases to 66.2%, suggesting that higher concentrations may have a detrimental effect on the encapsulation process. At low concentrations (L1, 0.2%), the encapsulation efficiency was 56.84%, which is expected as lecithin alone, without the optimal concentration of phenolic compounds, provides limited capacity for encapsulation. As the phenolic extract concentration increased to 0.4% (L2, 63.99%) and 0.6% (L3, 69.04%), there was a noticeable improvement in EE. This increase can be attributed to the better integration of phenolic compounds into the lipid bilayer, as higher concentrations offer more phenolic molecules for interaction with the liposomes, improving encapsulation efficiency (Popovici et al., 2024; Fathi et al., 2023). This behavior aligns with findings from other studies that report increased encapsulation efficiency with moderate concentrations of bioactive compounds. However, at concentrations of 0.8% (L4, 69.73%) and 1% (L5, 66.2%), the EE either plateaued or slightly decreased. The slight increase in EE at L4 can be explained by the near-saturation of the lipid bilayer with phenolic compounds, resulting in a relatively stable liposomal formulation. But when the

concentration reached 1% (L5), a decrease in EE occurred, likely due to aggregation or precipitation of the phenolic compounds, which disrupts the encapsulation process (Barekat et al., 2024; Kar et al., 2023). This trend is consistent with previous studies on liposomal formulations, where high concentrations of active compounds often lead to reduced encapsulation efficiency due to aggregation or instability in the liposomal structure (Hasibi et al., 202; Thiruvalluvan et al., 2024). Overall, the results suggest that the optimal encapsulation efficiency occurs at concentrations of 0.6% to 0.8% phenolic extract, where the phenolic compounds are effectively integrated into the liposomal bilayer without causing excessive aggregation or structural disruption. Beyond this range, EE tends to decrease, highlighting the importance of optimizing phenolic concentrations in liposomal formulations. These findings are in line with other studies that demonstrate the best encapsulation efficiency is achieved at moderate concentrations of bioactive compounds.

CONCLUSION

This study highlights the potential of grape pomace (GP) as a sustainable source of bioactive polyphenolic compounds. The combined use of soaking and ultrasound-assisted extraction (UAE) proved to be a highly effective strategy, significantly enhancing the recovery of phenolics and antioxidant activity compared to individual methods. Encapsulation of the extracted polyphenols in lecithin-based liposomes demonstrated promising results, with high encapsulation efficiency, stability, and favorable particle size, making them suitable for improved bioavailability and controlled release. The findings underscore the value of integrating advanced extraction and encapsulation techniques to overcome the challenges of bioactive compound stability and utilization. These results pave the way for the development of functional foods, nutraceuticals, and pharmaceuticals from grape pomace, contributing to its valorization and the promotion of circular bioeconomy practices.

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Table 1. Antioxidant activity (AA) and Total phenolic content (TPC) of grape pomace phenol extract (GPPex) using soaking and ultrasonication-assessed extraction techniques

Extraction Technique	GPPex (%)	AA (mg TE/ g DM)	TPC (mg Gallic acid/g DM)
Soaking	2	161.71±1.86	34.52±0.88
	4	169.25±1.22	42.39±3.28
	6	178.20±4.36	48.15±2.03
	8	192.23±2.15	52.80±3.75
	10	203.92±5.32	61.46±3.28
Ultrasonication	2	181.54±1.32	54.37±2.84
	4	196.09±3.37	63.65±2.58
	6	207.36±4.29	70.72±2.06
	8	214.41±1.03	76.35±1.80
	10	221.48±1.91	86.10±2.90
Soaking + Ultrasonication	2	229.59±0.63	73.61±2.07
	4	231.90±2.49	78.61±2.16
	6	237.64±2.18	84.30±1.94
	8	242.86±1.65	89.14±1.41
	10	250.76±2.25	101.10±1.75

Table 2. Particle size (nm), PDI and Zeta potential (mv) of liposome loaded grape pomace phenol extract

	Particle size (nm)	PDI	Zeta potential (mv)
lecithin (control)	105.8 ± 0.208	0.271 ± 0.007	-23.3 ± 1.00
L1	111.1 ± 0.46	0.262 ± 0.009	-19.7 ± 0.702
L2	84.14 ± 0.225	0.276 ± 0.012	-22.3 ± 1.42
L3	93.97 ± 0.96	0.253 ± 0.008	-22.3 ± 1.27
L4	89.62 ± 0.21	0.262 ± 0.001	-23.5 ± 0.96
L5	83.6 ± 0.34	0.255 ± 0.007	-23.3 ± 0.96

L1: lecithin with 0.2% grape pomace phenol extract, L2: lecithin with 0.4% grape pomace phenol extract; L3: lecithin with 0.6% grape pomace phenol extract; L4: lecithin with 0.8% grape pomace phenol extract; L5: lecithin with 1% grape pomace phenol extract

Table3. Encapsulation efficiency (EE %) of liposome loaded grape pomace phenol extract

liposomes	EE%
L1	56.84±1.90
L2	63.99±2.13
L3	69.04±0.76
L4	69.73±0.81
L5	66.20±1.01

L1: lecithin with 0.2% grape pomace phenol extract, L2: lecithin with 0.4% grape pomace phenol extract; L3: lecithin with 0.6% grape pomace phenol extract; L4: lecithin with 0.8% grape pomace phenol extract; L5: lecithin with 1% grape pomace phenol extract

الاستخلاص الأخضر والتغليف الليبوزومي لبوليفينولات ثفل العنب للاستخدام في المستحضرات الغذائية

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الملخص العربي:

يُعد ثفل العنب (GP)، وهو أحد المنتجات الثانوية لمعالجة العنب، غنيًا بالمركبات المتعددة الفينول ذات الخصائص المضادة للأكسدة والالتهابات والمضادة للميكروبات. تهدف هذه الدراسة إلى تعزيز استخلاص وتغليف البوليفينولات الموجودة في ثفل العنب لتحسين استقرارها وتوافرها البيولوجي. تم استخلاص البوليفينولات باستخدام مزيج تآزري من النقع والاستخلاص بمساعدة الموجات فوق الصوتية (UAE)، مما أدى إلى تحقيق أعلى نشاط مضاد للأكسدة (250.76 مجم TE/جم مادة جافة) ومحتوى فينولي إجمالي (101.10مجم GAE/جم مادة جافة) عند تركيز 10% من ثفل العنب. تم تحضير الليبوزومات التي تغلف هذه المستخلصات وتوصيفها لخصائصها الفيزيائية والكيميائية. أظهرت تركيبات الليبوزوم نطاق حجم الجسيمات من 83.6 إلى 111.1 نانومتر، وقيم جهد زيتا حول -23 مللي فولت، وكفاءة تغليف تصل إلى 69.73%. وتوضح النتائج أن الجمع بين النقع وUAS يزيد من استعادة البوليفينول في حين يوفر التغليف في الليبوزومات القائمة على الليسيثين نظام توصيل مستقر لهذه المركبات النشطة بيولوجيًا. ويؤكد هذا النهج على إمكانات تثمين ثفل العنب لتطوير الأغذية الوظيفية والمستحضرات المغذية.

الكلمات الاسترشادية: ثفل العنب, النشاط المضاد للاكسدة, مركبات فينولية, كفاءة الكبسلة.